

18. v. Wittich: 'Experimenta quaedam ad Halleri doctrinam de musculorum irritabilitate probandam instituta.' Königsberg, 1857, and 'Virchow, Archiv,' vol. 13, 1858, p. 421. In these papers, with the discovery of the excitation of muscle by distilled water, appears without doubt the first fact which overthrew the old theory of the equal irritability of muscle and nerve.

19. E. du Bois-Reymond: 'Gesammelte Abhandlungen zur allgemeinen Muskel- und Nervenphysiologie,' vol. 2, p. 700.

20. Kühne: 'Verhandlungen des Naturhist.-medizinischen Vereins zu Heidelberg,' Neue Folge, vol. 4, pp. 4, 5.

21. G. Retzius: 'Biologische Untersuchungen,' 1881.

A. Rollett: "Untersuchungen über den Bau der quergestreiften Muskelfaser." 'Wien, Akad. Denkschr.,' vol. 49, 1885.

22. 'Dr. H. G. Bronn's Classen und Ordnungen des Thierreiches, neu bearbeitet von O. Bütschli.' Leipzig und Heidelberg, 1888, vol. 1, p. 1298.

23. Kühne: "Neue Untersuchungen über motorische Nervenendigung." 'Zeitschr. Biol.,' vol. 23, pp. 88-95.

24. A. van Gehuchten: "Étude sur la structure intime de la cellule musculaire striée." 'La Cellule,' vol. 2, p. 289.

25. E. Hering: 'Sitzber. der k. Akad. zu Wien,' vol. 85, Abth. 3, 1882, p. 237.

26. Kühne: "Secundäre Erregung vom Muskel zum Muskel." 'Zeitschr. Biol.,' vol. 24, p. 383.

The drawings, figs. 1, 2, 3, 5, 8 are taken from the papers of Dr. K. Mays: "Histophysiologische Untersuchungen über die Verbreitung der Nerven in den Muskeln" ('Zeitschr. Biol.,' vol. 20, p. 449), and "Ueber Nervenfaservertheilungen in den Nervenstämmen der Froschmuskeln" ('Zeitschr. Biol.,' vol. 22, p. 354); figs. 9-13, from the author's work in 'Zeitschr. Biol.,' vol. 23, pp. 1-148, Plates A-Q.

"Contributions to the Chemistry of Chlorophyll. No. III." By
EDWARD SCHUNCK, F.R.S. Received June 19,—Read June
21, 1888.

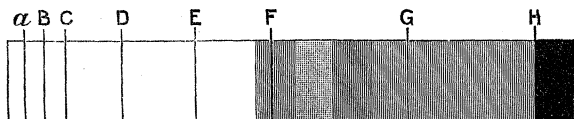
Products of the Action of Alkalis on Phyllocyanin.—In the first part of this memoir I gave a general account of the action of alkalis on phyllocyanin ('Proceedings,' vol. 39, p. 355). I shall now proceed to give the results obtained on further examining the products due to this action. The description of the products appearing in the first stage of the process of change induced by alkalis forms the subject of the present communication.

The great trouble involved in preparing any considerable quantity of phyllocyanin in a state of purity made it desirable to find out a method, if possible, of obtaining the products of the action of alkali directly from chlorophyll itself. The object in view was attained by acting on chlorophyll first with alkali and then with acid, thus reversing the process previously adopted and at the same time leading to the discovery of several new and interesting compounds, the formation of which had not been anticipated.

The plan I have pursued is as follows:—Fresh leaves—I prefer grass with broad blades to any other material—are exhausted with boiling spirits of wine containing from 80—82 per cent. of alcohol. The green extract is filtered hot, and being allowed to stand for a day or two away from the light, yields a dark-green voluminous deposit, containing chlorophyll mixed with fatty and other matters. This deposit is filtered off for further treatment, the pale-green filtrate being rejected. The green mass on the filter is now to be treated with a boiling solution of caustic soda in strong alcohol, which dissolves it in part. The insoluble portion is filtered off, and after washing with alcohol appears almost white.* Through the dark-green filtrate a current of hydrochloric acid gas is then passed until it acquires a strong acid reaction. The liquid first becomes yellowish-green, but after some time the colour changes to a dull purplish-green, and small crystalline needles arranged in stars, purple by reflected and dull-green by transmitted light, begin to appear on the

* Minute sparkling red crystals are always found interspersed in the amorphous mass of which the residue left by alcohol for the most part consists. These crystals are the chrysophyll of Hartsen, the erythrophyll of Bougarel, a very beautiful substance, which may be freed from the impurities accompanying it in this case in the following manner:—The residue, after washing with alcohol, is treated in the cold with chloroform, which dissolves the chrysophyll, leaving the greater part of the fatty matter behind. The yellow solution is filtered, mixed with a considerable quantity of alcohol, and left to stand for a day or two in the dark, when it deposits crystals of chrysophyll mixed with fatty matter. The deposit is filtered off, and placed, without removal from the filter, in a hot water funnel; here it is treated with a little hot glacial acetic acid. This removes all the fatty, along with some colouring, matter. The residue is dissolved in a little chloroform, and the solution, having been mixed with several times its volume of absolute alcohol, is left to stand in the dark. The next day a quantity of chrysophyll will have separated in crystals with a golden lustre and of a deep orange or red colour by transmitted light. The substance is rapidly bleached on exposure to air. In order to preserve it unchanged, it should, after filtration and rapid drying, be put into a glass tube through which a current of hydrogen is passed before sealing, then kept in the dark. According to Arnaud ('Compt. Rend.' vol. 102, p. 1119, and vol. 104, p. 1293), chrysophyll is identical with carotin. There can be no doubt that it contributes to the obscuration at the blue end of the ordinary chlorophyll spectrum; I have found it accompanying chlorophyll in all leaves that I have examined. Its solutions when sufficiently dilute show two broad bands at the blue end, without the least trace of absorption at any other part of the spectrum (fig. 1).

FIG. 1.



Absorption Spectrum of Chrysophyll.

sides of the glass. These needles continue to increase in quantity for some time; they are filtered off, washed with alcohol, and then treated with boiling ether, which removes a quantity of fatty matter, at the same time dissolving some of the substance itself. The residue is dissolved in a small quantity of chloroform, and the solution which is deeply coloured is then mixed with several times its volume of absolute alcohol. On standing, the liquid deposits a quantity of long crystalline needles, which are collected on a filter and washed with alcohol, in which they are only slightly soluble. The substance thus obtained is apparently an ethyl compound, and is probably the ethyl ether of the product formed by the action of alkalis on phyllocyanin, this being the conclusion to which its reactions seem to point. In mass it appears of a fine purplish-blue, and shows a semi-metallic lustre. Under the microscope it is seen to consist of acicular crystals, which are mostly opaque, but when very thin are transparent, and appear pale olive-coloured by transmitted light. It softens at 205°C. , but it has no definite melting-point. When strongly heated in a glass tube it is decomposed without yielding any crystalline sublimate, leaving a voluminous charcoal; heated on platinum it burns away without leaving any ash. It is insoluble in water, sparingly soluble in boiling alcohol and ether, more easily soluble in benzol and carbon disulphide, and very easily soluble in chloroform. The solutions when diluted have a dull-purplish or pink colour, and show an absorption spectrum identical with one already depicted as belonging to one of the derivatives of phyllocyanin ('Proceedings,' vol. 42, Plate 1, fig. 13). It dissolves in boiling glacial acetic acid and crystallises out on cooling. It is also soluble in concentrated hydrochloric acid, giving a solution which has the same greenish-blue colour, and shows the same absorption-bands as a solution of phyllocyanin in the same menstruum, but on the addition of water it is precipitated unchanged. The quantity of the product, in a crude state, obtained by the method described, amounted to 4.5 parts from 1000 of dry grass.

When methylic alcohol is employed in the extraction of leaves, and the same process as that above described is gone through, a similar compound is obtained, but differing from it in some respects. It crystallises in lustrous purple needles, rather lighter in colour than those from ethylic alcohol; it has no definite melting point; it is hardly soluble in boiling alcohol or ether, but easily soluble in chloroform, the solution showing the same absorption-bands as that of the other compound. It can hardly be doubted that this is the corresponding methyl ether.

These compounds are insoluble in aqueous alkalis, and are very little changed by prolonged boiling therewith, but on treatment with alcoholic potash or soda they are immediately dissolved and decomposed. The process is apparently one of saponification, the product

being the substance of which the compounds are the ethyl and methyl ethers respectively. In order to obtain this product the ethyl compound is treated with boiling alcoholic soda, in which it readily dissolves. The solution on standing deposits a sodium compound in the shape of a dark-green, almost black, semi-crystalline mass, which is filtered off, washed with absolute alcohol, and dissolved in water. The dark-green solution gives with acetic acid, of which a great excess must be avoided, a green flocculent precipitate, which is filtered off, thoroughly washed with water, and dissolved in ether. On slow evaporation the ethereal solution yields lustrous purple crystals, which must be separated before the solution has quite evaporated, for if there be any free acid present this will after most of the ether has evaporated, begin to act on the substance, inducing a change to which I shall allude presently.

The substance thus prepared is identical with that formed directly by the action of alkali on phyllocyanin, but by the process just described it is obtained in a state of much greater purity than by the direct method. Having read nearly everything that has been written on the chemistry of chlorophyll, I have come to the conclusion that this substance has never previously been described, and I think myself entitled therefore to give it a name. I propose to call it *Phyllotaonin* (from *ταῦν*, a peacock).

Properties of Phyllotaonin.—On spontaneous evaporation of its ethereal solution, it is obtained in regular flattened crystals or crystalline scales, which by reflected light appear of a fine peacock or steel-blue colour; the crystals are mostly opaque, but when very thin they are transparent and then appear brown by transmitted light. It melts at 184° to a brown resinous mass, but partial decomposition results from fusion, since the melted mass is no longer entirely soluble in chloroform, a little carbonaceous matter being left undissolved. Heated on platinum it swells up, giving off much gas and leaving a voluminous coal which burns away without residue; heated in a tube it swells and is charred without giving any perceptible sublimate. Phyllotaonin is insoluble in boiling water. It is easily soluble in boiling alcohol and ether, but it does not crystallise out on the solutions cooling; the solutions have the same colour, and show exactly the same absorption-bands as solutions of phyllocyanin, but if the least trace of any acid be present in the solution the spectrum gradually changes, the third band from the red end becoming fainter, while the fourth band as well as the first splits up into two. It is soluble in benzol and carbon disulphide, and very easily soluble in chloroform and aniline, but insoluble in ligroin. Phyllotaonin is easily soluble in glacial acetic acid, giving a solution of a fine violet colour, which shows a spectrum differing from that of the ethereal solution, and by this means it may be at once distinguished from

phyllocyanin, which dissolves in ether and in acetic acid, both solutions having a dull green colour, and showing the same spectrum. It is also soluble in concentrated hydrochloric acid, the solution having a bright bluish-green colour.

In contact with acids phyllotaonin undergoes a series of changes, accompanied by corresponding changes in the absorption spectrum. If to an ethereal or alcoholic solution of phyllotaonin a small quantity of an acid, such as hydrochloric, sulphuric, oxalic, tartaric or acetic acid be added, the colour of the solution changes slowly from green to brown, and now shows the spectrum frequently referred to in which two bands, that in the red and that in the green, are seen split up into two (see fig. 11 of the Plate previously referred to). A further change takes place on standing, one of the bands in the green becoming darker, the other lighter (see spectrum, fig. 12). Here the action stops with all the acids named except acetic acid. On treating phyllotaonin with boiling glacial acetic acid it dissolves, and the dark purple solution if sufficiently concentrated deposits on cooling crystalline needles, arranged in fan-shaped masses. These collected on a filter and dried show a fine purple colour, and closely resemble the supposed ethyl-compound of phyllotaonin; its solutions show the same absorption spectrum as the latter. This product is doubtless a compound with acetic acid; stronger acids such as sulphuric or hydrochloric acid yield no similar compounds. The products formed by the action of acids may in all cases be re-converted into phyllotaonin by means of alkali. The process of re-conversion may be traced in its course with the crystallised acetate. If the latter be treated with aqueous potash in the cold it dissolves; acetic acid added gives a green precipitate which dissolves in ether, the solution showing the spectrum of fig. 11, but if boiling alcoholic potash be employed, the corresponding ethereal solution shows the spectrum of phyllotaonin. Under the influence of acetic acid the latter again passes through the series of changes previously described. That the changes induced on the one hand by acids, and on the other by alkalis, are due in one case to hydration and in the other to dehydration, seems probable. After being heated to the melting point, phyllotaonin gives solutions showing the spectrum, fig. 12, but by treatment of the fused substance with alcoholic potash it returns to its original state. It is difficult to attribute the change in this case to anything but loss of water, the latter being taken up again on treatment with alkali.

A potassium compound of phyllotaonin is obtained on adding potash to an alcoholic solution of the substance; it crystallises in needles which are purple by reflected light. The sodium compound obtained in the same way is hardly crystalline. A boiling alcoholic solution of phyllotaonin to which cupric acetate and acetic acid have

been added, deposits on cooling and standing a quantity of crystalline needles arranged in pretty rosettes which, after filtering off and drying, appear bluish-green by reflected as well as by transmitted light, and show no metallic lustre; the alcoholic solution of this compound shows the same absorption spectrum as that of the corresponding phyllocyanin compound, and, like the latter, it is not decomposed nor in any way changed by treatment with boiling hydrochloric acid. Similar compounds containing iron and silver may be obtained, but their properties are not sufficiently interesting to merit detailed description; they resemble the corresponding phyllocyanin compounds.

On adding metallic tin to a solution of phyllotaonin in hydrochloric acid and allowing to stand, the solution soon loses its bright bluish-green colour, and becomes olive-green, finally reddish-yellow. Water now gives a red precipitate, which filtered off and washed dissolves in alcohol with a crimson colour, the solution showing a spectrum similar to that of the final product of the action of tin and hydrochloric acid on phyllocyanin.

Though there can be little doubt as to the purple crystals formed by the action of hydrochloric acid on an alcoholic solution of alkaline chlorophyll being an ether, I have not succeeded in reproducing it by the direct action of acid on an alcoholic solution of phyllotaonin. The solution retains its bluish-green colour unchanged, deposits no crystals even on long standing, and gives with water a precipitate consisting of uncombined phyllotaonin. A compound resembling that in the purple crystals may, however, be formed from phyllotaonin by a different process. If to an alcoholic solution of phyllotaonin ethyl iodide and a little caustic potash be added, the solution on boiling deposits a small quantity of a black powder, which being collected on a filter and treated with dilute acid, is found to be soluble in alcohol, ether, and chloroform, giving purple solutions which show the same spectrum as solutions of the purple crystals. It is, however, easily soluble in aqueous alkali, and may therefore be a mono-ethyl, the other being a di-ethyl ether. It is probably identical with the compound formed directly from phyllocyanin by a similar process, as described in the first part of this memoir, in the solutions of which the spectrum (fig. 13) so frequently referred to was first observed. This very peculiar spectrum belongs, it appears, to four distinct compounds.

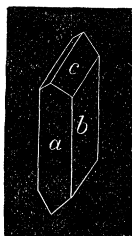
In order to explain the formation of the purple crystals by the process above described, we may suppose that by the influence of alkalis chlorophyll is first converted into a substance which by decomposition with acids yields phyllotaonin, and this in the nascent state and in contact with alcohol and hydrochloric acid undergoes etherification.

Of the compounds above described I have analysed such as were

well crystallised and appeared to be pure, but I will not give the results until I have had an opportunity of confirming them with freshly prepared material. The difficulty experienced in preparing sufficient quantities of pure substances from chlorophyll has proved a great drawback in this investigation and has much retarded its progress.

My friend Dr. Burghardt, of the Owens College, has had the kindness to examine at my request the crystalline form (fig. 2) of phyllotaonin, and reports as follows :—

FIG. 2.



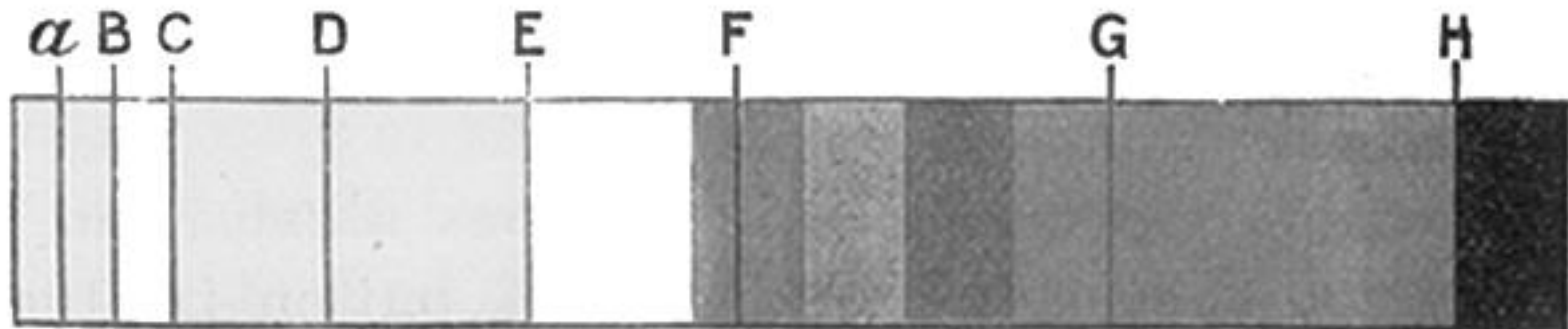
Crystal system monosymmetrical, oblique rectangular prism, formed by the combination of the ortho- and clino-pinacoids.

The terminal faces are a negative hemipyramid. The faces *b* (010 Miller or $\alpha P\alpha$ Naumann) predominate, giving a "vertical tabular habit" to the crystal.

It was impossible to obtain any measurements of the angles owing to the smallness of the crystals and the roughness of the faces. The value, therefore, of the hemipyramid indices is unknown. The faces "*a*" are 100 (Miller) or $\alpha P\alpha$ (Naumann), whilst the faces "*c*" are the negative hemipyramid $-hhl$ (Miller) or $-mP$ (Naumann).

They cleave parallel to the ortho-pinacoid distinctly. Examined in polarised light they exhibit depolarisation, on rotating the Nicol's prism the colour changing from a light-yellow to a rich brownish-red.

FIG. 1.



Absorption Spectrum of Chrysophyll.

FIG. 2.

